Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 11-20 are pending in the application. The specification was amended to update the priority information, to comport the description of the drawings in the specification with the formal drawings, and to add the address for the ATCC. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

The paragraph beginning on page 1, line 3:

This application is a divisional of U.S. Appl. No. 08/891,640, filed July 11, 1997; said 08/891,640 claims priority to U.S. Appl. No. 60/021,247, filed July 12, 1996, the disclosures of both of which [is] are herein incorporated by reference.

The paragraph beginning on page 8, line 10:

Figure 1(a-[b]n). The nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of the transcriptional intermediary factor-2 (TIF2) protein. This protein has a deduced molecular weight of about 160 kDa. The amino acid sequence of the functional coactivator TIF2.1 protein fragment is shown from amino acid residue 624 to residue 1287.

The paragraph beginning on page 9, line 27:

Figure 3(a-[b]c). Amino acid sequence of TIF2: homology with SRC-1 indicates the existence of a novel family of NR mediators.

The paragraph beginning on page 10, line 1:

(a-b) Alignment and amino acid sequences of TIF-2 (SEQ ID NO:2) and the steroid receptor coactivator SRC-1 (SEQ ID NO:3) (Onate, S.A. *et al., Science 270:*1354-1357 (1995)). Two charged clusters rich in acidic and basic amino acid residues, three serine/threonine (S/T)-rich regions and one glutamine-rich region are highlighted. The N-terminal charged cluster contains putative bipartite nuclear localization signals (NLSs)

(overlined). The regions encoding TIF2.1 (amino acids 624 to 1287; functional coactivator fragment) and dnSRC-1 (amino acids 865 to 1061; dominant negative fragment) are indicated. An asterisk identifies the TIF2 stop codon. Note that TIF2.1 and dnSRC-1 do not overlap, indicating that dnSRC-1 may possibly contain a NR-interacting region distinct from that of TIF2.1.

The paragraph beginning on page 10, line 12:

[(b)] (c) Schematic comparison of TIF2 and SRC-1. Percent identities (similarities in parentheses) of homologous regions are indicated. The N-terminal charged cluster harbo[u]ring the putative NLS and the C-terminal S/T-rich region of TIF2 are not, or only weakly, conserved in SRC-1.

The paragraph beginning on page 13, line 13:

Figure 6 (a-[b]c). Schematic representation of reporter genes (A) and receptor expression vectors (B-C) (see the Materials and Methods section of Nagpal *et al., EMBO J 12(6):*2349-2360 (1993) for a detailed description of construction). Sequences of mCRBPII (SEQ ID NO:11) and mCRBPII(17m-ERE)/CAT (SEQ ID NO:11) are indicated. Minus and plus numbers are with respect to the RNA start site (+1). In (B-C), the various regions (A-F) of wild-type RARs and RXRs, as well as their truncation mutants, substitution mutants and chimeric receptor constructs are schematically represented (not to scale) (see Zelent *et al., Nature 339:*714-717 (1989); Leid *et al., Trends Biochem. Sci. 17:*427-433 (1992); Leid *et al., Cell 68:*377-395 (1992); Nagpal *et al., Cell 70:*1007-1019 (1992); and Allenby *et al., Proc. Natl. Acad. Sci. USA 90:*30-34 (1993)). Numbers indicate the amino acid positions

in the wild-type receptor. The positions of the amino acid substitutions are indicated with an arrow.

The paragraph beginning on page 15, line 8:

Figure 8(a-[e]f). Mapping of the TIF2 nuclear receptor interacting domain (NID).

The paragraph beginning on page 15, line 25:

(e-f) Effect of TIF2 NID point mutations on stimulation of NR AF-2 activity. Cos-1 cells were cotransfected with 1 μg of the $(17m)_5$-TATA-CAT reporter, 0.2 μg of GAL-hERα(EF) or GAL-mRXRα(DE), and 2.5 μg of the TIF2.1 wildtype or mutated fragments, as indicated. The reporter gene activation relative to the TIF2.1 wildtype activity and in presence of 10^{-6} M estradiol (E2) or all-*trans-retinoic* acid (RA), respectively, is indicated for each mutant (black bars); for comparison, *in vitro* binding of the respective mutants relative to TIF2.1 wildtype binding in presence of ligand is indicated by the white bars. Each bar represents the mean value obtained from at least three (interaction) or at least four (transactivation) experiments, respectively; standard deviations are indicated. Note that the absolute values for TIF2.1 wildtype activity varied by ±16% when cotransfected with GAL-hERα(EF) and by ±34% when cotransfected with GAL-mRXRα(DE). In the *in-vitro*-interaction assays, the affinity of the TIF2.1 wildtype standard varied by less than ±25%. Expression levels of TIF2 mutants in the cells were verified by Western blot (not shown) with mouse monoclonal antibody 3Ti3F1, which is directed against an epitope outside the mutated area.

The paragraph beginning on page 20, line 25:

In another aspect, the invention provides isolated nucleic acid molecules encoding the TIF2 polypeptide having an amino acid sequence as encoded by the cDNA clone deposited as ATCC Deposit No. 97612 on June 14, 1996 (American Type Culture Collection, (ATCC) [Rockville, MD] 10801 University Boulevard, Manassas, Virginia 20110-2209). The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) or the nucleotide sequence of the TIF2 cDNA contained in the above-described clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated nucleic acid molecules, preferably DNA molecules, are useful as probes for gene mapping by *in situ* hybridization with chromosomes and for detecting expression of the TIF2 gene in human tissue, for instance, by Northern blot analysis.

Claims 1-10 were cancelled.